

## ABSTRACT

The disclosed invention relates to general and specific methods to use the Primer Extension/Nick Translation (PENT) reaction to create an amplifiable DNA strand, called a PENTAmers. A PENTAmers can be made for the purpose of amplifying a controlled length of DNA located at a controlled position within a DNA molecule, a process referred to as Positional Amplification by Nick Translation (PANT). In contrast to PCR, which amplifies DNA between two specific sequences, PANT can amplify DNA between two specific positions. PENTAmers can be created to amplify very large regions of DNA (up to 500,000 bp) as random mixtures (unordered positional libraries), or as molecules sorted according to position (ordered positional libraries). PANT is fast and economical, because PENTAmers preparation can be multiplexed. A single PENTAmers preparation can include very complex mixtures of DNA such as hundreds of large-insert clones, complete genomes, or cDNA libraries. Subsequent PCR amplification of the preparation using a single specific primer can positionally amplify contiguous regions along a specific clone, along a specific genomic region, or along a specific expressed sequence.